A history of cocaine self-administration alters normal phasic dopamine activity in the nucleus accumbens during first order conditioning for natural rewards

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Abstract

The nucleus accumbens (NAc) of the mesolimbic system plays an essential role in associative learning. Previous research has shown that neurons in the NAc encode stimuli predictive of rewards and that dopaminergic activity in this region is important for using this predictive information to guide behavior. More specifically, phasic dopamine activity in the NAc has been shown to correlate with the learning of associations between stimuli and natural rewards. However, it is unknown how previous cocaine exposure affects this activity. In order to test this, we used fast-scan cyclic voltammetry (FSCV) in the NAc to measure the release of dopamine in response to stimulus and reward presentation during first order conditioning. In first order conditioning, animals were repeatedly presented both with a stimulus that predicted a reward and with one that predicted no reward. We found that cocaine-exposed animals were able to behaviorally associate stimulus and reward to the same degree as water-administering controls. However, phasic dopamine released in the NAc in response to reward-predictive stimuli was significantly lower in cocaine-exposed animals with than in controls. Furthermore, there was differential dopamine release to the two types of stimuli in controls, but not in cocaine-exposed animals. These results suggest that abnormal dopamine signaling in animals with a history of cocaine abuse does not impair their ability to learn simple associations between stimulus and reward. In the future, FSCV might be used to determine if the activity of phasic dopamine in the NAc accounts for the inability of cocaine-exposed rats to learn more complex, higher order associations.

It is essential to be able to use features of the environment as predictors of food and other primary needs; therefore, associations between these features and these primary, or natural, rewards are a necessary part of the survival of all animals. Associative learning involves the formation of a mental link between previously unrelated stimuli so that the presence of one stimulus evokes the characteristics of the paired stimulus. For instance, by repeatedly exposing an animal to an auditory stimulus that precedes the receipt of a contingently delivered food reward, the animal will learn that the stimulus predicts the reward and update its behavior to reflect this.

In order to form associations between environmental stimuli and rewards, there must be a neural mechanism that works to integrate sensory and motivational information and subsequently effectuate changes in motor output. The nucleus accumbens (NAc) in the mammalian midbrain has been shown to be a crucial structure in this respect. Activity in the neurons that project to the NAc has been implicated in motivated behaviors that lead to the acquisition of primary rewards (Kelley, 2004; Roitman, Stuber, Phillips, Wightman, & Carelli, 2004). Also, environmental cues that precede a primary reward in time have been shown to cause an increased firing pattern of neurons in the NAc that correlates with an animal's learned prediction of the reward from the presence of a cue (Day, Roitman, Wightman, & Carelli, 2007). On a related line, lesions of the NAc in rats impaired their ability to show normal increases in latency of responding to aversive stimuli (Schoenbaum & Setlow, 2003). This finding shows that the NAc is also essential for using associations between cues and aversive stimuli to guide behavior so as to avoid such stimuli in the future. Furthermore the fact that the NAc encodes information about both rewarding and aversive stimuli suggests that it is really tracking information about motivationally salient stimuli regardless of valence.

The subregions of the NAc and the surrounding regions that are connected neurally play an important role in associative learning. There are two anatomical areas of the NAc: the core and the shell. These two areas differ with respect to their functional properties and inputs from and outputs to other brain regions (Zahm and Brog 1992). For instance, it has been suggested that the core is important for encoding information about cues and response contingencies, whereas the shell is important for using this information to affect behavior (Saddoris, Stamatakis, & Carelli, 2011). In addition to the NAc, the orbitofrontal cortex (OFC) and basolateral amygdala (BLA) are regions which have displayed important roles in associative learning. These two regions appear to form a circuit in conjunction with the NAc that mediates associative learning and value-guided decision making (Schoenbaum, Roesch, & Stalnaker, 2006). It has been shown that contralateral disconnections of the BLA and the NAc prevent higher order conditioned responses, e.g. the association of a light to a tone that was previously associated with a primary reward, which suggests that the circuitry between these regions is important for processing motivational value (Setlow, Holland, and Gallagher, 2002). It has also been suggested that the interconnection between the OFC and BLA is important for the integration of specific environmental information with more general value information which then synapses into the NAc (McDannald, Lucantonio, Burke, Niv, and Schoenbaum, 2011). From this, it can be seen that the NAc receives information from other brain regions that is crucial to the process of associative learning. Due to its major role in this greater brain circuitry of working to integrate reward, motivation, drive, and motor information, the NAc is a highly important structure to study with regard to associative learning.

The results of Carelli, Ijames, & Crumling (2000) suggest that there are discrete populations of neurons in the NAc that encode information about different types of reward, such

as food and cocaine. Thus, the neurons that encode information about cocaine may be selectively strengthened by cocaine abuse, resulting in aberrations in natural associative learning processes. However, before the effects of cocaine on associative learning are expounded upon, the neuropharmacological nature of associative learning must be explored.

The activity of the neurotransmitter dopamine in the NAc has been shown to track important features of associative learning. The ventral tegmental area (VTA) in the midbrain projects dopamine neurons to the NAc (Ikemoto, 2007). Stuber, Klanker, de Riddler, Bowers, Joosten, Feenstra, & Bonci (2008) found that innervation of VTA dopamine neurons was transiently strengthened as a result of associative reward learning. More importantly, dopamine transients (sub-second concentration spikes) in the NAc have been shown to shift dynamically in time from the presentation of a primary reward to the presentation of a cue predictive of the primary reward, indicating the NAc's possible role in the formation of the simple associations that are essential to an animal's survival (Day et al., 2007). The link between associative learning and dopaminergic activity in these mesolimbic systems has been further examined with learning based on models of prediction error.

Prediction error results when an animal is surprised by the delivery or absence of a stimulus that it did not predict to follow a particular event (Schultz, Dayan, & Montaque, 1997). For example, when initially presented with a lever, a rat will not press that lever to receive a primary reward. However, when successively paired with a primary reward such as food, the lever becomes associated with the characteristics of the food and its value changes from neutral to positive. The rat learns that its prediction of a neutral value to the lever was incorrect and subsequently alters its behavior by pressing it when it is presented. There is a learning model that describes this prediction error and helps to elucidate the role of phasic dopamine activity in

associative learning. This temporal difference reinforcement learning (TDRL) model states that learning is continuous, with the animal making predictions and having their errors revealed at all times (Schoenbaum and Niv, 2008). Increased phasic dopamine activity in the NAc may signal a prediction error that a rat learns to associate with a primary reward. For instance, a reward-predictive cue elicits increasing transients as learning progresses while the transients elicited by the primary reward that follows the cue decrease (Day et al., 2007). TDRL models maintain this hypothesis because when the rat has completely learned that the cue is predictive of the reward, receiving the reward will no longer generate a prediction error and thus a dopamine transient at the time of receipt.

The TDRL model can be used to understand the phenomena of first and second order conditioning, which in turn carries implications for the role of dopamine in learning. In first order conditioning, a neutral stimulus (CS) is associated with a stimulus (primary reward, or UCS for brevity) that elicits an innate response. As mentioned above, after repeated pairings of the CS followed by the UCS (in which the delivery of the latter is contingent on the presentation of the former), the CS alone begins to evoke similar responses from the animal to acquire the UCS that were evoked previously solely by the UCS. These responses can be those of autoshaping, where the animal engages in orienting and approach behaviors that were originally elicited by the UCS alone. The approach behavior of interest in the present study is the entry of a foodcup, accomplished by poking the nose into the foodcup to acquire a reward following the presentation of the CS. Related to first order conditioning is the phenomenon of second order conditioning. This is where a neutral stimulus (CS2) is associated to a different stimulus (CS1) that has already been associated with the delivery of a UCS and thus has acquired motivational value. After repeated pairings of the CS2 followed by the CS1, the CS2 begins to evoke similar

responses from the animal to acquire the UCS that were evoked previously solely by the CS1 alone. For example, through learning, money becomes associated with items such as television sets, which are associated to the primary reward of entertainment. TDRL states that an animal will recognize prediction errors at all times. This is consistent with the second order model wherein an animal can realize a prediction error while experiencing the CS2 preceding the CS1, even in the absence of the UCS or the acquisition response (Schoenbaum and Niv, 2008). Thus, like the case where an animal is surprised by the delivery of the UCS following the initially neutral CS, an animal should likewise be surprised by the presentation of the now motivationally-significant CS1 following that of the neutral CS2. The NAc should therefore signal a prediction error in both cases, resulting in the updating of behavior. However, the similarity of the CS2-CS1 association and the CS1-UCS association has been disputed. It has been shown that devaluing either the CS1 or the UCS has no affect upon the ability of the CS2 to evoke the conditioned response required to obtain the UCS (Holland and Rescorla, 1975). This implies that learning involving second (or higher) order associations is different from learning involving first order associations. In fact, it is unlikely that the formation of the CS2-CS1association involves the encoding of a direct mental representation of the UCS; rather, the CS2 is likely associated with the CS1 in virtue of the motivational value it acquired from association with the UCS (Holland and Rescorla, 1975). Thus, second and higher order learning likely involve a more abstract processing of cues than does first order conditioning. This presents the question of how phasic dopamine release in the NAc looks in higher order versus first order conditioning. However, this question cannot be answered by the present study due to time constraints. Despite this, the relevance of higher order conditioning to the present study remains:

dopamine signaling and prediction error functions may operate more simplistically in first order conditioning than in higher order conditioning.

It was mentioned above that the NAc learning system might be modified resulting in a failure to encode harmful stimulus properties; for instance, drugs of abuse such as cocaine might access this system and disrupt natural reward processing, resulting in the irrational, habitual behaviors characteristic of drug addiction (Wise, 1995). Thus, associative learning is likely impaired by cocaine abuse. Moreover, the prediction errors generated by dopamine transients in the NAc are a casualty of cocaine abuse (Redish, 2004). Whereas primary rewards produce dopamine transients only if unpredicted, cocaine self-administration produces dopamine transients directly (Stuber, Wightman, & Carelli, 2005); thus, after extended pairings of a cue with a cocaine reward, NAc dopamine transients will occur to the cue, but will still be released at reward consumption (Redish, 2004). This notion was corroborated by the finding that dopamine transients were still evoked by self-administration of cocaine after repeated pairing with a temporally prior audiovisual stimulus (Phillips, Stuber, Heien, Wightman & Carelli, 2003). This finding contrasts with the results found by Day et al. (2007) and suggests that cocaine abuse interferes with the error prediction system in the NAc, since the dopamine transients giving rise to error prediction signaling are not shifting dynamically in time to one event. This suggestion presents a host of problems concerning associative learning in rats and humans alike. First, TDRL holds that an association between a cue and a reward is completely learned when the receipt of the reward no longer evokes a prediction error (as it is completely predicted by the cue) and thus should not evoke a dopamine transient. Since the cocaine reward *does* evoke a dopamine transient, it appears that it is not being completely predicted by the cue. Likewise, Redish (2004) says that an animal that is abusing cocaine will over-select actions that result in

the receipt of the drug; this is because the animal is continuously having its reward expectation violated by getting a false prediction error in terms of dopamine at the reward receipt. The animal will increase the value of the state that led to this false prediction error, i.e. the instrumental response that yielded the cocaine reward, and thereby increase the value of future cocaine use. Since this prediction error is false and unable to be corrected due to the direct action of cocaine on dopamine neurons, the value of cocaine use will eventually increase to a level higher than its real value. This will lead the animal to respond more for cocaine than a nondrug alternative even if the alternative is more rewarding than the cocaine. If the prediction error, and therefore the dopamine transient, were at a negligible level upon the receipt of the cocaine reward, then the animal would not have to change its behavior as the cue-reward association would be completely learned. Thus, cocaine abuse results in the aberration of the natural process whereby prediction errors enable an animal to consume future rewards in proportion to their actual value. This is one means by which cocaine abuse may lead to irrational decision-making, i.e. by assigning higher value to future cocaine use despite the availability of more rewarding alternatives.

It is unclear if this specific aberration in instrumental responding seen in cocaine-abusing rats will carry over to subsequent conditioning wherein the primary reward is not a drug of abuse such as cocaine. Redish's theory applies to situations where there is a choice between cocaine and a nondrug alternative (Ahmed, 2004). However, we are concerned with the situation where the only available reward is a conditionally presented sucrose pellet. Yet, given what the theory entails, the cocaine-abusing animal will value nondrug use lower than drug use and should therefore have a blunted response to the presentation of sucrose pellets during first order conditioning. Thus, since the natural process of environment-reward association is disrupted by

cocaine abuse when other alternatives are available, it seems that this disruption will affect other types of associative learning, e.g. learning to discriminate between two different cues on the basis of contingent reward presentation. Indeed, it was found that rat pups that were given prenatal injections of cocaine failed postnatally to exhibit first order conditioning compared to controls (Heyser, Chen, Miller, Spear, and Spear, 1990). However, adult cocaine abuse does not impair first order conditioning (Saddoris, Cameron, Briley, and Carelli, 2010). This presents the question of how dopamine signaling and thus error prediction function in the NAc in cocaine-exposed animals. The present study will therefore be aimed at discovering how a history of cocaine abuse affects the activity of phasic dopamine in the NAc.

Given that dopamine shifts dynamically in time from the UCS to the CS1, it is probable that a similar kind of shift may take place from the CS1 to the CS2; however, the present study will be unable to verify this empirically. More pertinent to the present study is the question of how phasic dopamine will shift from the UCS to the CS in a rat with a history of cocaine abuse. It is currently unknown how this shift is affected by cocaine abuse as well as how TDRL would calculate prediction errors for a cocaine-abusing rat performing first order conditioning. At least three possibilities exist for the former. The first is that the phasic dopamine generated in response to the reward-predictive CS will be the same for cocaine-exposed rats as it is for normal rats. If this is the case, then the behavioral deficits seen in cocaine-exposed rats may not be due to dopamine signaling in the NAc, but rather to how the NAc detects and interprets the dopamine.

The second and third possibilities involve a problem with dopamine signaling in the NAc: the phasic dopamine generated in response to the reward predictive CS will be either higher or lower in the cocaine-exposed rats than in the normal rats. If the phasic dopamine is higher, then this might indicate that cocaine abuse has sensitized the rat to cues predictive of

reward, thus leading it to value the cue more than a normal rat would. On a neuropharmacological level, this may be due to an upregulation of dopamine receptor proteins brought on by repeated cocaine-related dopamine activity. This second scenario is the possibility in greatest congruence with TDRL. As TDRL states, the animal should use prediction errors to change its behaviors in order to optimize its benefits. Indeed, if rats attribute more value to rewards due to sensitization, then we would expect more phasic dopamine in response to cues predictive of these rewards as learning progresses. However, if phasic dopamine released in response to the cue is lower in cocaine-exposed rats after they learn the cue-reward association, this might indicate that they are not using prediction errors to optimize their benefits from rewards. If phasic dopamine release is lower in this case, then it does not shift completely from the reward, thus indicating that the rat may not be fully predicting the reward from the cue. This is consistent with Redish's (2004) idea that cocaine-exposed rats may be over-selecting states that lead to cocaine consumption over those that lead to nondrug consumption, even when the nondrug is more rewarding. If the value attributed to states predictive of nondrug rewards is not to scale with the actual value of those rewards, then we can expect less than normal amounts of dopamine in response to cue onset.

In order to measure phasic dopamine in the NAc during first order conditioning, a technique called fast-scan cyclic voltammetry (FSCV) will be employed. FSCV allows for subsecond dopamine release in the NAc to be recorded with great accuracy (Robinson, Venton, Heien, & Wightman, 2003). Thus, the dopamine transients that occur close in time to the presentation of the CS and the receipt of the UCS will be able to be accurately quantified. However, before FSCV will be used to determine the phasic dopamine profiles of rats during first order conditioning, the ability of the rats to perform first order conditioning must be shown

and inculcated in the laboratory. This is because on the FSCV test day we will be unable to determine whether or not an individual rat learned the task without already having established its proficiency at it.

The present experiment seeks to know whether dopamine signaling in the NAc is responsible for the learning anomalies exhibited by rats with a history of cocaine abuse. Specifically, can we use the phasic dopamine profiles of these rats to track their unimpaired discrimination between cues? To test this, we will use FSCV to record real-time dopamine release while the rats learn a simple first order task. We hypothesize that one of three possibilities of phasic dopamine activity in cocaine-exposed rats will be shown to be the case: (1) phasic dopamine will be released in response to a reward-predictive cue to the same degree as it is in controls, (2) this release will be higher than in controls, or (3) this release will be lower than in controls. Each possibility holds implications regarding the effects of drug abuse on learning and how they might be dealt with pharmacologically.

Method

Subjects

Experimentally naïve male Sprague-Dawley rats (n = 21) weighing approximately 300 g at the time of arrival were used. The rats were individually-housed and allowed to habituate to the vivarium for approximately one week, during which time they had *ad-libitum* access to food and water and were maintained on a 12 hour light/dark schedule. They underwent a surgery to implant a chronic catheter into the jugular vein which was the conduit for subsequent cocaine delivery. After a one week post-surgery recovery, the rats were shifted to food restriction that maintained their weight at 85% of their normal, free-feeding weight. Rats were divided into two

main conditions: cocaine abusing (n = 9) and controls (n = 12). Controls were further divided into those rats which received an infusion of saline via the implanted catheter (n = 7) and those which received non-abusing doses of cocaine through the implanted catheter (n = 5). All animal procedures were conducted in accordance with the National Institutes of Health Guidelines for the Care and Use of Laboratory Animals and the guidelines of the University of North Carolina at Chapel Hill Institutional Care and Use Committee.

Materials

All lever-press training and behavioral training took place in a custom-built behavioral chamber (43 x 43 x 53 cm; MED Associates, St Albans, VT, USA) located in a sound-reducing cabinet. The interior walls of the cabinet were covered in metal mesh to provide insulation from external electrical signals. Each chamber was illuminated by a houselight located on the ceiling. Two different versions of the chamber were used in the present experiment: one for lever-press training and the other for behavioral training. The chamber for lever-press training included one retractable lever (Coulbourn Instruments, Whitehall, PA, USA), which was four centimeters from the floor of the chamber. The lever caused the rat to be infused with a solution (0.33 mg/dL) of cocaine and heparinized saline while the other did nothing. A mechanical bus was used to deliver the cocaine (housed in a syringe) through a tube lining and into the rat's catheter. The chamber for behavioral training included a digital camera mounted on the ceiling that enabled digital recording on a computer (api Software) enabling us to check if the rat was unresponsive to cues or was sleeping at any time. A foodcup (approximately four centimeters above the floor) was mounted in the center of the right wall of the chamber. The behavioral training chamber had two levers similar to the ones in the lever-press training chamber. Visual

cues delivered in the behavioral training chamber consisted of either a flashing light or a solid light delivered by lights mounted above the levers.

During FSCV recordings, the rats were connected to a recording harness that terminated in a headstage consisting of two cannulas and one stimulation fiber, all secured into place with dental cement (Plexon Inc., Dallas, TX, USA). The harness was connected at the other end to a commutator (MED Associates and Crist Instruments), which allowed for free movement throughout the chamber during recording sessions. During the recording sessions, dopamine signals from the NAc were passed to a high definition cyclic voltammetry (HDCV) program. A separate computer controlled the presentation of the first order cues and recorded the number of foodcup entries/exits and percent time spent in foodcup (TRANS IV, MED Associates). NeuroExplorer software (NEX Technologies, Littleton, MA, USA) was used to further analyze the behavioral data and HDCV Analysis was used to further analyze the NAc dopamine data.

Design and Procedure

The rats first underwent a surgery in which a chronic catheter was implanted into the jugular vein; this allowed cocaine to be administered directly into the bloodstream. Rats were prepared for surgery by the intramuscular or intraperitoneal injection of an anesthetic consisting of ketamine hydrochloride (100 mg per kg body weight) and xylazine hydrochloride (10 mg per kg body weight). Each rat was given one week to recover from the surgery, after which it began lever press training. The training was conducted in two-hour sessions once a day for 14 consecutive days. The goal of the training was to get rats to self-administer cocaine by learning that cocaine delivery was contingent on the pressing of a lever. Control rats received an infusion of saline upon lever pressing. After lever press training, the rats underwent a second surgery to

prepare them for FSCV recording. As in the catheter surgery, rats were prepared for surgery by the intramuscular or intraperitoneal injection of an anesthetic consisting of ketamine hydrochloride (100 mg per kg body weight) and xylazine hydrochloride (10 mg per kg body weight). Before surgery began, each rat was secured into a stereotaxic frame that prevented movement of the head. Two cannulas and one electrical stimulation electrode were then implanted into the brain. One cannula was implanted into the NAc while the other was implanted into the left forebrain for reference purposes. The electrical stimulation electrode was implanted into the ventral tegmental area (VTA) in order to stimulate dopamine neurons there to release dopamine into the NAc. The rats were given one week to recover post-surgery. After a total of 30 days had passed after the last day of lever press training, the rats were started on behavioral training. The first part of this was ten days of first order conditioning. During this, the rats learned to associate a flashing light (CS+) with a contingently delivered sucrose pellet (primary reward or UCS). There was also a solid light (CS-) that was not reinforced by sucrose pellets. These two different types of CS were used as conditions in order to test whether or not rats could discriminate between them. Normal rats are able to discriminate between reinforced (CS+) cues that predict reward and non-reinforced (CS-) cues that do not, responding more to the former (Day, Wheeler, Roitman, & Carelli, 2006). Thus, the use of two different types of CS provided us with a measure of the learning impairments caused by cocaine abuse. On the tenth day of conditioning, FSCV was performed on the rats in order to determine the profile of dopamine transients in the NAc in response to the behavioral events. In FSCV, the potential of an electrode is ramped linearly versus time. This potential ramp allows for dopamine molecules near the electrode to be oxidized and a characteristic current subsequently recorded. The detection of this current indicates the amount of phasic dopamine released in the NAc. Electrochemical data was

collected via a glass-sealed carbon-fiber electrode inserted into the right cannula and lowered into the NAc. A reference electrode was inserted into the left cannula for the purpose of providing a stable voltage to which the analytical electrode was referenced. Voltammetric recordings were made every 100 milliseconds and allowed for the identification and measurement of analytes pertinent to the present study (dopamine and pH) at the subsecond level.

Results

All rats learned the cue-food associations, as shown by increased entries into the foodcup during cue presentation across sessions; there was a main effect of Day, $F(8, 96) = 5.033$, $p < 0.001$. This main effect was due to a lower number of foodcup entries for both groups on day 1 than on all subsequent days. There was no significant Day X Condition interaction, $F(8, 96) = 0.727$, $p = 0.668$, showing that on a given day both cocaine and saline rats entered the foodcup to the same degree. In addition to the main effect of Day, we observed a main effect of Cue, $F(1, 12) = 10.100$, $p < 0.01$, showing that there were more foodcup entries overall to the CS+ than to the CS-. This suggests that, in general, rats were able to discriminate between the CS+ and the CS-. However, there was no significant Cue X Condition interaction, $F(1, 12) = 0.433$, $p = 0.523$, showing that both cocaine and saline rats entered the foodcup to the same degree in response to a given cue type.

To better understand the rats' discrimination between the CS+ and the CS-, we looked at the significant Day X Cue interaction, $F(8, 96) = 3.936$, $p < 0.001$. The significance of this interaction shows that there was a greater increase in foodcup entries across the 10 days for the CS+ than there was for the CS-. For the controls and the cocaine-exposed rats, there was a

significant difference between CS+ and CS- entries on days 5-10, showing that both groups were able to discriminate between the cues by day 5 of conditioning. However, since there was no significant Day X Cue X Condition interaction, $F(8, 96) = 0.214$, $p = 0.988$, this increase was not held preferentially by one group over the other. Observing day 10 for both groups, we found there to be a significant difference between CS+ and CS- foodcup entries with controls, $p < 0.05$, and cocaine-exposed rats, $p = 0.05$.

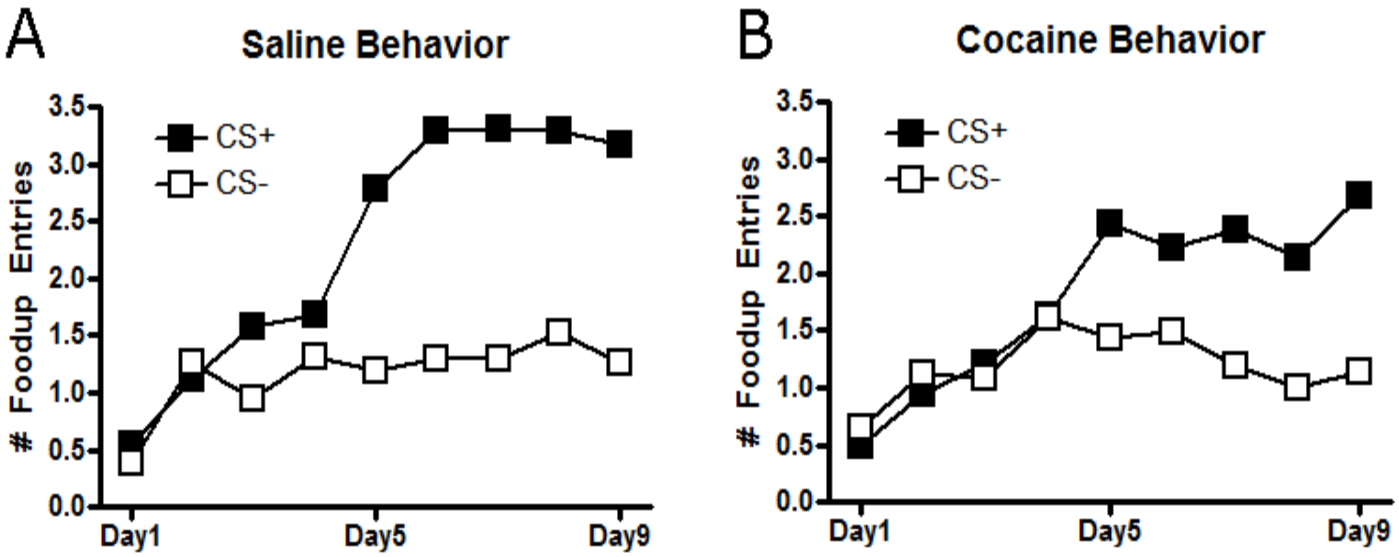

Fig. 1. Behavioral results for days 1-9 of first order conditioning. (A) Saline controls learned the significance of the CS (CS+ and CS-) during conditioning. By day 5, they were able to discriminate between the two cues, as shown by the rapid increase in CS+ foodcup entries relative to CS- foodcup entries from day 4. On days 5-9, there was a significant difference in foodcup entries in response to the two cues, $p < 0.05$. (B) Cocaine rats learned the significance of the CS (CS+ and CS-) during conditioning. By Day 5, they were also able to discriminate between the two cues, as shown by the rapid increase in CS+ foodcup entries relative to CS-

foodcup entries from day 4. On days 5-9, there was a significant difference in foodcup entries in response to the two cues, $p < 0.05$

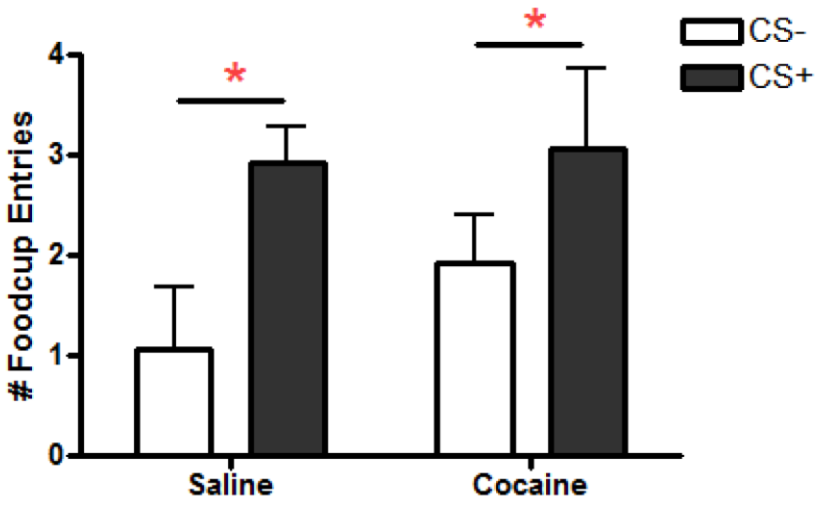

Fig. 2. Behavioral results for day 10 of first order conditioning. There was a significant difference between CS+ and CS- foodcup entries in both the saline and cocaine groups, *$p \leq 0.05$.

Rapid DA release was recorded in the NAc using fast scan cyclic voltammetry (FCSV) when the task was well learned. We found that cocaine exposure dramatically affected dopamine signaling in the NAc. Specifically, there was significantly less overall dopamine release in cocaine animals compared to saline controls during the cue period analyzed, as revealed by a main effect of Drug, $F(1, 7) = 11.49$, $p < 0.02$. Indeed, it was further found that drug exposure also interacted with dopamine release dynamics during the different cues. For example, there was a significant trend towards an interaction of Drug X Cue, $F(1,7) = 7.29$, $p < 0.05$, which post hoc tests indicated that dopamne release in the cocaine animals was not different between CS+

and CS-, ($p = 0.38$), while in saline animals, there was more dopamine release to the CS+ than to the CS- ($p < 0.03$). Indeed, there was significantly more dopamine release in the saline animals than cocaine animals selectively during the CS+ cue ($p < 0.005$), but there were similar levels of dopamine release during the CS- periods, ($p = 0.33$).

In order to analyze the temporal dynamics of dopamine release in the NAc, the baseline levels of dopamine were compared with the levels of dopamine in response to cue presentation. We compared the level of dopamine in each 100 ms bin 1-20 to the level of baseline dopamine (bin 0) for each cue (CS+ and CS-) within each group (cocaine and control). We found that the overall level of dopamine release following cue onset for the cocaine-exposed animals was significantly greater than baseline for bins 2-20 during the CS+ periods, ($p < 0.05$), and for bins 1-13 during the CS- periods, ($p < 0.05$). Similarly, the overall level of cue-evoked dopamine for the controls was significantly greater than baseline for bins 1-20 during the CS+ periods, ($p < 0.001$), and for bins 1-13 during the CS- periods, ($p < 0.0001$).

To further explore the temporal dynamics of the effects of Drug and Cue on NAc dopamine levels, we examined the dopamine release in each bin to understand the significant interaction of Drug X Cue X Bin, $F(20, 140) = 3.92$, $p < 0.00001$. We looked at how the bins differentially affected the two-way interaction of Drug X Cue. For the cocaine animals, there was no significant difference between the CS+ and CS- in terms of dopamine concentration for all bins 1-17, i.e. for 1.7 seconds after either cue was presented. In contrast, for the controls, there was a significant difference between the CS+ and CS- in terms of phasic dopamine in every bin except for bins 3 and 4, i.e. between 0.2 and 0.4 seconds after either cue was presented. The bins in which there was the largest significant difference, ($p < 0.0001$), in dopamine levels between cue types within the control group were those located 0.5-2.0 seconds after either cue was

presented. Such an effect of bin did not occur for the cocaine group, as the only significant difference in dopamine between cue types occurred between 1.7 and 2.0 seconds after either cue was presented. These results show how the dopamine release dynamics during the 2-second period after cue presentation resulted in a Drug X Cue interaction, as there was significantly more dopamine release to the CS+ than the CS-, ($p < 0.0001$), during most of this period for the controls, whereas such a significant difference existed for only a brief period of time (0.3 seconds) for the cocaine-exposed rats.

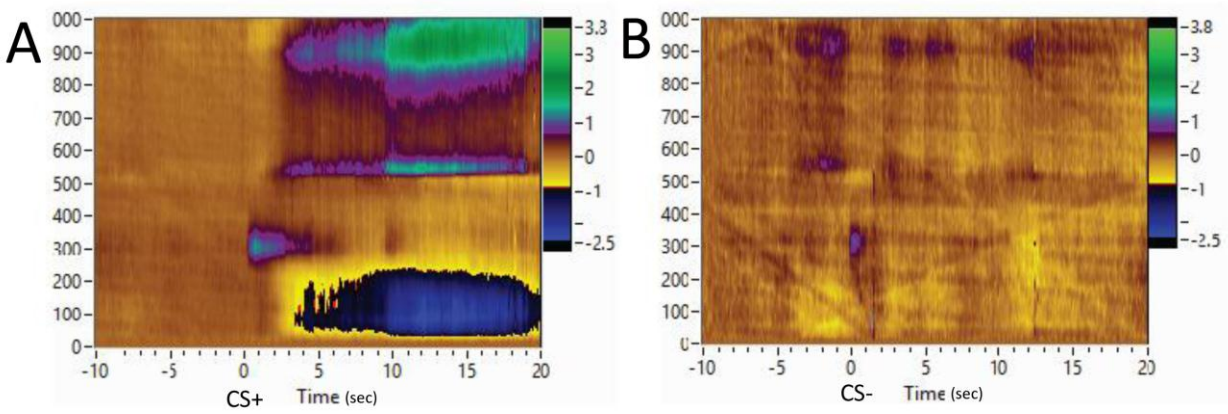

Fig. 3. Color plot of electrode current in nA versus time for representative saline rat during day 10 of first order conditioning. At time = 0 seconds, the cue (CS+ or CS-) is presented, resulting in a rapid rise of phasic dopamine (represented as a purple-green peak (~-3.3 nA) at point number = 315). (A) The dopamine peak at the presentation of the CS+ is much more pronounced than that in (B) for the CS-. This suggests that the rat had a greater prediction for the primary reward after the CS+ presentation than after the CS- presentation.

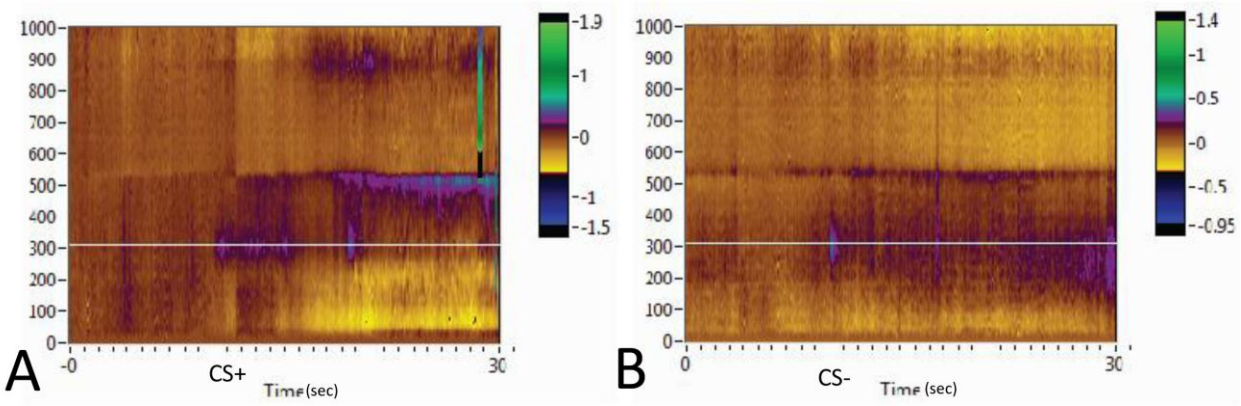

Fig. 4. Color plot of electrode current in nA versus time for cocaine-exposed rat during day 10 of first order conditioning. At time = 0 seconds, the cue (CS+ or CS-) is presented, resulting in a rapid rise of phasic dopamine. (A) The dopamine peak at the presentation of the CS+ is not significantly different from that in (B) at the presentation of the CS-. This suggests that the rat had an equal prediction for the primary reward after both the CS+ and CS- presentations.

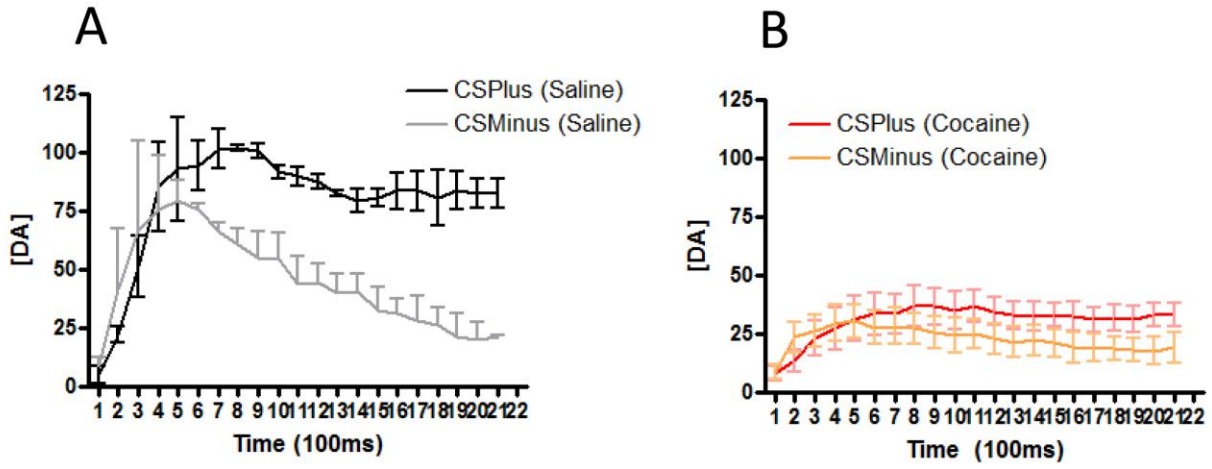

Fig. 5. Plots of [dopamine (DA)] vs. time for the 2.2 seconds after the cue was presented. (A) contained the average [DA] vs. time for n = 2 saline recordings and (B) contained that for n = 7 cocaine recordings. In (A) and (B), the cue was presented at time = 100 ms. In (A), there was a significant difference between CS+ [DA] and CS- [DA] across the 2.2 seconds, while there was no such significant difference in (B).

Discussion

The results of the present study found that cocaine-exposed animals were able to discriminate between reward-predictive cues and non-reward-predictive cues to the same degree as controls. Thus, no behavioral deficits were observed in animals with a history of cocaine abuse, consistent with Saddoris et al. (2010). However, it was also shown that phasic dopamine released in the NAc in response to reward-predictive cues was significantly lower in animals with a history of cocaine self-administration than in water-administering controls. Further, there was differential dopamine release to the cues for the controls, but not for the cocaine-exposed animals.

The results obtained from FSCV show that dopamine signaling in cocaine-exposed animals differs significantly from that in normal animals. Most notably, there was differential dopamine release to the cues in the control animals, but not in the cocaine-exposed animals. Cocaine-exposed animals had significantly less overall phasic dopamine release than controls during the 2.2 second cue period (regardless of cue type). That this effect was not mediated by cue type suggests that there may have been a downregulation of dopamine neurons in the NAc resulting from cocaine exposure. Since cocaine blocks the reuptake of dopamine and therefore increases the amount of synaptic dopamine present during cocaine administration, the number of firing dopamine neurons needed to attain the same level of neurotransmitter would be reduced. This plasticity-induced change in neuronal architecture may have persisted into first order conditioning, wherein animals did not have access to cocaine. On a motivational level, this may have led the cocaine-exposed animals to undervalue any cue that was not predictive of a drug reward. This is consistent with Redish's (2004) model which suggests that cocaine-exposed animals overvalue states that lead to drug rewards due to a constant, uncorrected prediction error

generated at the receipt of the reward. However, given the highly significant Drug X Cue interaction and the normal behavioral discrimination of the cocaine-exposed animals, it is unlikely that the sort of downregulation just proposed had any meaningful effect on learning.

It may seem odd that phasic dopamine released to the CS- is significantly higher than baseline in both groups since one would not expect it to generate prediction errors via reward prediction; however, previous research (Day et al., 2007) has shown that this phenomenon occurs across first order conditioning. This may serve the purpose of using cues with similar physical characteristics as a guide to reinforcement. Additionally, since the two cues types possessed similar characteristics, stimulus generalization may have occurred to a degree after extended conditioning, partly accounting for the dopamine response to the CS-. Furthermore, in both groups, phasic dopamine generated in response to the CS- returned to baseline 1.4 seconds after cue onset; phasic dopamine generated in response to the CS+ was also above baseline in both groups for the whole cue period. This shows that dopamine was released to the CS+ for a longer period of time than it was for the CS-, an effect that was independent of group type. A possible explanation for this is that right after CS- onset, the visual system realized that the solid light is in fact not the flashing CS+, resulting in the correction of reward prediction by ceasing the release of phasic dopamine. This finding supports the idea that a degree of stimulus generalization may have occurred.

Examining the behavioral data, it is clear that cocaine-exposed animals were able to perform first order conditioning to the same degree as controls. This parity of performance is evidenced by the absence of any effects of Drug on the amount of foodcup entries. After noting the absence of Drug effects, a pairwise comparison showed that, on each day of training, there were no significant differences in CS+ or CS- foodcup entries between groups. Furthermore,

Figure 1 shows that both groups learned the cue discrimination on day 5 of conditioning and maintained it for the remainder of training. These results show that cocaine-exposed animals were able to discriminate between cues just as well as controls, but also that their learning progressed as nearly the same rate as controls. If cocaine-exposed animals were unable to discriminate between the cues, then the behavioral data would be in line with the earlier extrapolation of Redish's (2004) model: because cocaine-exposed animals over-select for states leading to drug receipt, they may subsequently undervalue states leading to nondrug receipt such as entering the foodcup in response to CS+ presentation. However, discrimination and therefore learning did take place in the cocaine-exposed animals and was behaviorally indistinguishable from the learning seen in controls.

A further analysis of the voltammetric data suggests that prediction errors were reported differently in cocaine-exposed animals than in normal animals, which has implications regarding the effects of cocaine-abuse on associative learning. If phasic dopamine provides prediction-related signaling that is necessary for learning, then we might expect for it to be released in cocaine-exposed animals in the same way as it is in controls, for it seems *prima facie* that the cocaine-exposed animals learned the same information about the first order task as controls. However, if the prediction error hypothesis is true, then it seems that the cocaine-exposed rats could not have learned the same information. This is because there was a significant Drug X Cue interaction and significantly less dopamine release in the cocaine-exposed animals than in controls selectively during the CS+ cue. These findings, together with the idea that phasic dopamine provides essential prediction-related signaling, present the idea that phasic dopamine in the NAc may be responsible for some other aspect of learning in addition to first order conditioning. For instance, some minimal amount of phasic dopamine may be necessary for the

learning of cue-reward associations, with the blunted amount seen in cocaine-exposed animals still meeting this condition. If this is the case, then it may be that a blunted phasic dopamine response only affects learning when animals engage in more complex higher order learning processes. The implications of abnormal dopamine signaling for higher order learning are discussed below.

The limitations of the present study deal with shortcomings in the behavioral and voltammetric components. Due to the uncharacteristic performance by some of the controls on the first order conditioning task, e.g. showing discrimination on one day but no discrimination the next, there was accompanying uncharacteristic variance that affected the results. In addition, the foodcups used in the training boxes had smaller-than-usual detection ranges for nosepokes (as compared to Saddoris et al., (2010)). As a result, there may have been different sensitivity to detect of foodcup entries in response to cue and reward presentation than were reported previously. The voltammetric shortcomings deal with the difficulty in administering a successful recording using FSCV during a behavior session. Inserting the electrode into the NAc is possibly the most difficult part of recording; many electrodes are broken in the process and therefore many recording opportunities are lost. Also, during recording, movements by the animal in the box can damage the electrode. These limitations are evidenced by the small amount of successful recordings in the present study (n = 9). Furthermore, we possess voltammetric data for only two control animals, whereas we have voltammetric data for seven cocaine-exposed animals. This small amount of voltammetric data for the controls due to electrode complications limits our power in ascertaining a significant difference in phasic dopamine between controls and cocaine-exposed animals.

A form of higher order learning that may be impaired by cocaine abuse is second order conditioning. This is conditioning wherein a neutral cue is repeatedly paired with a cue that has acquired motivational value in virtue of its association with a reward. Eventually, this neutral cue will be associated with the other cue and thereby come to elicit characteristics of the reward. Thus, a second order association is learned. Cocaine exposure has been shown to impair this form of conditioning while leaving intact the ability to perform first order conditioning (Saddoris et al., 2010). The present study suggests that the blunted phasic dopamine response in cocaine-exposed animals may account for these deficits in second order conditioning. Although cocaine-exposed rats performed normally in first order conditioning, there may have been crude encoding or abnormal processing of cue-related information by the NAc compared to controls. This diminished encoding and/or processing may allow for simple first order associations to be learned, but may disrupt the learning of more complex second order associations. As discussed above, prior cocaine exposure may preserve the amount of phasic dopamine release necessary for first order learning, but prevent it from reaching the amounts necessary for second order learning. However, a more likely alternative obviates talk of a *necessary* amount of phasic dopamine required for learning. This alternative account holds that cocaine exposure may lead to diminished encoding of associations involving outcome value, such as those between cues and rewards, while preserving associations between cues and *responses* made to obtain rewards. These latter associations do not directly involve the value of the rewarding outcome that follows cue presentation; rather, they arise due to a certain cue being repeatedly paired with an instrumental response, such as the entering of a foodcup. Indeed, previous research has shown that prior cocaine exposure preserves the formation of these types of associations while impairing those involving outcome value, evidenced by the maintenance of responding for

reward after reward devaluation in cocaine-exposed rats (Schoenbaum and Setlow, 2005). This idea that cocaine exposure preserves cue-response learning may account for the normal performance of cocaine animals in first order conditioning. Indeed, the normal foodcup entries made to the CS+ may have been due to strong cue-response associations despite weak cue-reward associations.

The idea that cocaine exposure impairs only the formation of associations involving outcome value gives insight into how it may impair second order conditioning. Second order conditioning likely involves more complex associations than does first order conditioning, as no reward is presented during the former type of conditioning. Thus, the animal will have to use information about reward value gained in first order conditioning in a more complex way to guide this learning. Since cocaine-exposed animals are impaired at acquiring this type of information, then they are *a fortiori* unable to use it in the more complex way that second order conditioning requires. The present study suggests that the blunted phasic dopamine response seen in cocaine-exposed animals may be the neurological underpinning of their impaired performance in second order conditioning. If phasic dopamine provides error signaling that corrects predictions about expected outcome value, then it may be a necessary component in the formation of cue-reward associations. Thus, if phasic dopamine release during conditioning is abnormally low, then cue-reward associations are at best crudely formed, resulting in impaired second order performance later on. In order to test this idea, FSCV might be used in conjunction with first and second order conditioning to determine if and how phasic dopamine shifts from first to second order cues in second order conditioning. This would enable us to better understand the effects of cocaine abuse on error prediction. Also, optogenic techniques allowing the control

of individual neuron firing could be used to determine whether or not phasic dopamine activity in the NAc causes learning or merely an artifact that is *caused by* learning elsewhere in the brain.

Using features of the environment to predict the availability and delivery of rewarding experiences is vital to the survival of all animals. Cocaine abuse seems to leave intact the formation of some associations involving these features while impairing the formation of others. The present study suggests two explanations for this: that simple associations between cue and reward are unaffected by abnormal dopamine signaling or that such signaling impairs these associations and thereby prevents them from being used in more complex ways. In the future we can test to see which of these is correct and whether or not this abnormal signaling is the cause of deficits seen in more complex higher order learning. This may aid researchers in the development of pharmacological intervention aimed at treating the behavioral and cognitive aspects of drug abuse and addiction.

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